

Lot No.

Description

- LaboPass™ IP-*Pfu* PCR Premix is an optimized 2X PCR Master mix containing IP-*Pfu* DNA Polymerase, dNTPs, MgCl₂, reaction buffer, loading dye and stabilizers that is aliquoted into the Thin-Wall 8-strip PCR tube. This premix formulation simplifies PCR setup. The user simply adds template, primers, and DW to start the reaction.
- LaboPass™ IP-*Pfu* DNA Polymerase is a thermostable DNA polymerase cloned from *Pyrococcus furiosus* and a recombinant form expressed in *E.coli*. This archaeal polymerase possesses 3'-5' exonuclease proofreading activity together with 5'-3' polymerase activity, which allows high fidelity DNA amplification. *Pfu* polymerase remains its polymerase activity during extended exposure at 98°C unlike *Taq* polymerase. Therefore, this enzyme can be used to amplify difficult templates (e.g. high GC content or stable secondary-structure).

Specifications

Components	IP- <i>Pfu</i> Polymerase, dNTPs, reaction buffer, loading dye stabilizers
Type	Ready-to-use (Only DNA template and primers are needed)
Reaction volume	20 µl (2X PCR Master mix is aliquoted (each 10 µl) into the PCR tube)
Store at	-20°C

Storage and Stability

LaboPass™ PCR Premix is stable for 1 year when stored at -20°C. Repeated freezing and thawing of the premix is not recommended.

Applications

- High fidelity PCR
- Preparation of PCR products for cDNA cloning
- Site-directed mutagenesis
- Blunting of DNA ends

Quality Control

Each lot of IP-*Pfu* polymerase, reaction buffer and dNTPs is tested for contamination such as *E.coli* genomic DNA, nicking, endonuclease and exonuclease.

Standard Reaction

Components	Volumes (µl)
2X IP- <i>Pfu</i> PCR Premix	10 µl
Forward Primer (10~50 pmoles/µl)	1 µl
Reverse Primer (10~50 pmoles/µl)	1 µl
DNA Template (Variable*)	1~2 µl
Distilled water	6~7 µl
Total reaction volume	20 µl

* Amount of DNA template

- Eukaryotic genomic DNA 10-200 ng
- Prokaryotic genomic DNA 1-50 ng
- Purified homogeneous DNA <5 ng
(e.g. plasmid, lambda DNA, etc)
- cDNA : 0.5-10% of RT reaction volume

General Thermo-Cycler protocol

Step	Time	Temperature
Initial denaturation	1-5 min	94-95°C
25-35 Cycles:		
Denaturation	10-25 sec	94-95°C
Annealing	10-25 sec	45-70°C
Extension	60 sec/1 kb	68-72°C
Final extension	5 min	68-72°C

* Note

- Vortex all solutions and spin down carefully before using
- Dispense on ice and spin down again